Biological Pathways in the Development of Dupuytren's and Peyronie's Diseases

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Chapter 7

General discussion and future perspectives

This thesis consists of studies on the biological background of Dupuytren's disease (DD) and Peyronie's disease (PD), fibrotic disorders of the hand and penis, respectively.

The first aim of this thesis was to find out whether the Wnt genes reported by Dolmans et al. (2011, 2012) are dysregulated in Dupuytren's cords and nodules. In the genome-wide association study by Dolmans et al. nine different loci were found to be involved in genetic susceptibility of Dupuytren's disease. [1] Six of these loci contain genes that are involved in the Wnt signaling pathway: WNT2, WNT4, WNT7B, SFRP4, RSPO2, and SULF1. The WNT2 gene was also found to be associated with Peyronie's disease. [2] In a separate study, the association of WNT2, WNT4, WNT7B, SFRP4 and RSPO2 loci and Dupuytren's disease was confirmed. [3] Another study showed some additional genes related to Wnt signaling that are associated with Dupuytren's disease, namely WNT5A, EBPF2, SMAD3, and AXIN1. Gene expression analysis of WNT2, WNT5B and WNT11 in Dupuytren's disease showed no differential expression of WNT2, upregulation of WNT5B, and downregulation of WNT11 in primary disease tissue samples. [4]

Wnt signaling and fibrosis

Fibrosis is a normal response to injury necessary for ultimate functional recovery of the injured structure, but also a condition that may have major negative implications, as it may lead to organ failure and even subsequent death. In DD, an abnormal form of fibrosis formation takes place that – in a worst case scenario - leads to recurrent or uncorrectable contractures. In the genome-wide association study that aimed to encipher some of the genetic code for DD, we stumbled upon six areas in the genome related to Wnt signaling. The findings demanded further functional studies that are the subject of this thesis.
Numerous papers in the last 20 years have shown the contribution of Wnt signaling as well as aberrant collagen processing in many fibrotic disorders. [5-8] Apart from that, the Wnt signaling pathway is also essential for organ and tissue development. It consists of Wnt ligands that bind to transmembrane frizzled and LRP receptors. Dependent on Wnt regulation proteins, this may lead to a change in intracellular β-catenin degradation. In β-catenin dependent signaling, target gene transcription follows β-catenin translocation to the nucleus, where it binds to enhancers (Figure 1).

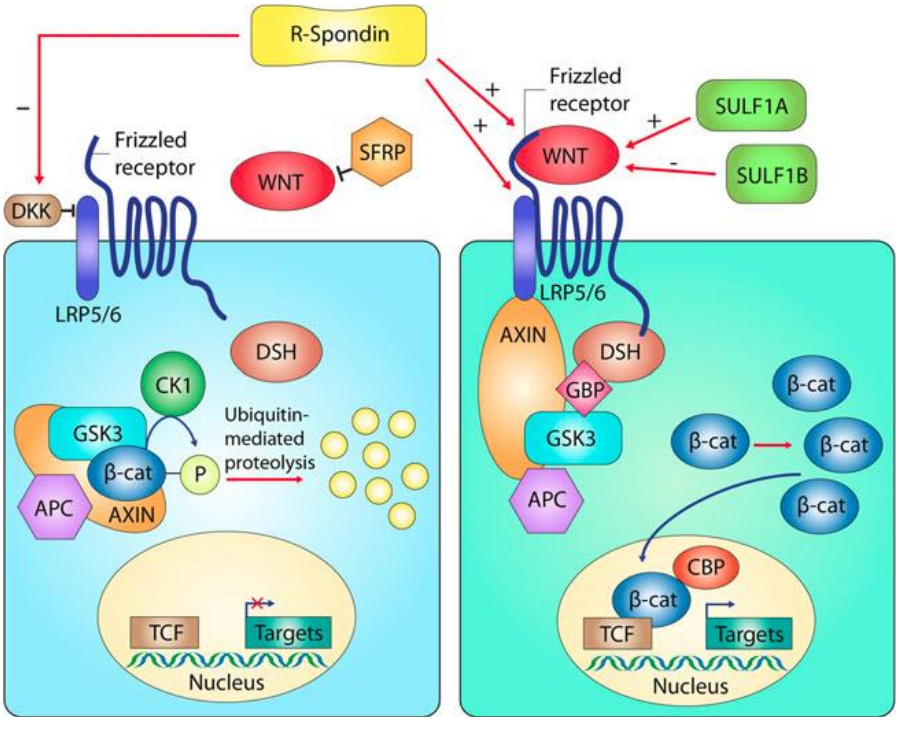

Figure 1. Schematic representation of β-catenin dependent Wnt signaling. Left: inactivated pathway. β-catenin is degraded, and forthcoming target genes are in a repressed state. Right: activated Wnt signaling, in which β-catenin degradation is reduced. Wnt signaling is repressed by SFRPs, by binding to either a Wnt or frizzled receptor. R-spondin positively regulates β-catenin signaling by interacting with the frizzled receptor and the low-density lipoprotein-receptor–related protein (LRP5/6) and by competing with the dickkopf protein (DKK). APC: adenomatous polyposis coli. CBP: cyclic AMP response-element–binding (CREB) protein–binding protein. CK1: casein kinase 1. DSH: disheveled protein. GBP: GSK3-binding protein. GSK3: glycogen synthase kinase 3. P: phosphorylation. SFRP: secreted frizzled-related protein. TCF: T-cell factor.

From: Ten Dam et al., Further evidence of the involvement of the Wnt signaling pathway in Dupuytren's disease; Dolmans et al., Wnt signaling and Dupuytren's Disease.

Several other Wnt pathways that are independent of β-catenin, make use of other receptors, such as ROR2 and RYK. [9] Since this β-catenin independent signaling has a larger diversity in receptors and co-receptors, these pathways are more context-dependent and complicated to describe. [10] The Wnt ligands that we mainly described, Wnt2, Wnt3a, Wnt5a, and Wnt7b, all belong to the group of 19 know Wnt proteins and coding genes in humans. [11] While Wnt3a is a classic β-catenin dependent ligand, Wnt5a is mostly involved in β-catenin independent signaling. [12] Among others, we also studied the SFRP4, WIF-1, and WISP-1 genes. These genes and proteins influence Wnt signaling in different stages of the pathway. Both SFRP4 and WIF-1 are known to inhibit Wnt signaling, thereby being anti-fibrotic, and WISP-1 is a target of an activated β-catenin dependent Wnt signaling pathway, known to be a strong pro-fibrotic regulator. [13-15]

At the beginning of this century, the first studies on Wnt in combination with fibrotic disorders were published, [5, 16] and in the following years, knowledge on the relation between Wnt and fibrosis expanded. The importance of β-catenin was revealed, although several factors, not just Wnt signaling, are able to adjust its activity. [17]

Fibrosis in major organs, such as the heart, lungs, kidneys, liver and skin, showed overlap in aberrant expression levels in humans. [9] Some of these genes were also found to be dysregulated in Dupuytren's and Peyronie's tissues, as shown in Table 1.

Since DD and PD are diseases with a relatively high prevalence, and both diseases share characteristics with more severe fibrotic disorders, these findings are interesting as it comes to a broader look at the combination and possibly even prediction of these diseases. Aberrant Wnt signaling and collagen processing have been described in other conditions as well, so our research might have clarified the knowledge on fibrosis and fibromatosis specifically. In the following text, I present a broader view on these subjects.

Organ/disease	Wnt signaling components	
	Up	**Down**
Lung	B-CATENIN, GSK3B, LEF1, LRP5, LRP6, WISP-1, WNT-1, WNT-3A, WNT-5A, WNT-7B, WNT-10B	DKK1
Kidney	B-ARRESTIN, DKK3, WNT-1, WNT-2, WNT-4, WNT-5A, WNT-9B, WNT-10B	WNT-6
Skin	B-CATENIN, DKK2, FZD2, LEF1, WIF-1, WNT-3A	DKK1, SFRP1
Liver	RSPO1, RSPO2, RSPO3, WNT-5A	
Heart	WNT-1, WNT-5A	
Peritoneal cavity	B-CATENIN, LEF1, WNT-1, WNT-5A	
Fibromatosis (DD and PD)	SFRP4, WISP-1, WNT5A, WNT7B	WIF-1, WNT2, WNT3A

Table 1. Wnt related genes dysregulated in fibrotic disorders, from previous and current research. Interpreted and supplemented from: *Burgy, Königshoff – The WNT signaling pathways in healing and fibrosis*

SUMMARY AND FUTURE PERSPECTIVES

Chapter 1 provides a general introduction and outline of this thesis.

In **Chapter 2** we measured mRNA levels of WNT2, WNT4, WNT7B , RSPO2, SFRP4 and SULF1 in cord and nodule tissue and compared this to control tissue (unaffected transverse ligaments of the palmar aponeurosis of Dupuytren patients). In nodules, we found significantly lower levels of WNT2 (nine-fold), and significantly higher levels of WNT7B (five-fold) and SFRP4 (two-fold) compared to unaffected tissue. In cords, higher mRNA levels of SFRP4 (two-fold) are seen compared to unaffected tissue. mRNA levels of WNT4, RSPO2 and SULF1 did not differ between affected and unaffected tissues. In addition, we stained for Wnt2, Wnt4, Wnt7b, Rspo2, Sfrp4 and Sulf1 in the same tissues. In agreement with mRNA levels, no significant differences were seen in staining for Wnt4, Rspo2, and Sulf1. Also in agreement with qPCR results, we found more Wnt7b staining in nodules than in controls. A discrepancy was found in the staining for Wnt2: significantly less staining was seen in cords but not in nodules. Remarkably, no differences were observed in Sfrp4 staining between affected and unaffected tissues.

Because of the relationship between Wnt signaling and β-catenin levels, we also stained for β-catenin. As mentioned above, increased mRNA and protein levels are seen for Wnt7b in nodules, but not in cords. Indeed, significantly increased cytosolic and nuclear β-catenin protein levels are seen in nodules but not in cords. In addition, protein staining of Wnt7b co-localized with β-catenin staining, and both proteins co-localized with staining for α-smooth muscle actin staining, a marker for myofibroblasts (i.e. activated fibroblasts). This intimate relationship points towards a role of Wnt7b as a pro-fibrotic effector molecule in Dupuytren's disease. A marked upregulation of Wnt7b has also been reported in idiopathic pulmonary fibrosis, especially in myofibroblasts of the fibroblastic foci. [18, 19] In this context it is of interest to note that overexpression of Wnt7b results in increased protein levels of Wnt5a, [20] and that the presence of Wnt5a is a necessity for the production of collagen by the strong pro-fibrotic cytokine TGF-β. [21] Interestingly, the WNT5A locus has also been associated with Dupuytren's disease. [4]

mRNA levels of Sfrp4 were upregulated in cords and nodules, but no differences were observed in Sfrp4 staining between normal and affected tissues. It has been found that increased levels of Sfrp4 results in an attenuation of fibrotic tissue in the hearth and in the kidney due to inhibition of the β-catenin dependent Wnt pathway. [14, 22] The fact that no increase was observed in Sfrp4 staining in nodules and cords, suggests that the activation of the β-catenin dependent Wnt pathway was not affected by Srp4.

We found a clear downregulation of WNT2 on mRNA and protein levels. As one of the Wnt ligands, Wnt2 is known to be a pro-fibrotic regulator. In skin fibrosis, the WNT2 gene was found overexpressed. [23] In fibroblasts from keloid scars, knockdown of β-catenin led to a decrease in Wnt2 expression. [24] Wnt2 shows different expression patterns in pancreatic cells associated with fibrosis. It seems to be overexpressed alongside β-catenin in moderate fibrosis, but diminishing in dense fibrotic tissue. [25] This finding might explain the downregulation we found in the most fibrotic areas of end-stage Dupuytren's nodules and cords. We suggest that Wnt2 is essential in the development of fibrotic tissue, but fades away when this process has been completed.

In **Chapter 3** we have extended our studies on whether there are alterations in Wnt signaling in fibrotic Dupuytren's tissues. We quantitatively examined the expression of 84 genes related to the Wnt pathway in nodules and in unaffected transverse ligaments of the palmar aponeurosis of 12 patients. 41

genes were differently expressed in nodules; of these 41 genes, 10 were significantly increased 2-fold or more and 14 were significantly decreased 2-fold or more. The four WNT loci found to be associated with Dupuytren's disease by means of genome-wide association studies, namely WNT2, WNT4, WNT5A and WNT7B, all show a different expression compared to control tissue. WNT2 and WNT4 were downregulated, whereas WNT5A and WNT7B were upregulated. The upregulation of WNT5A was verified with immunohistochemistry. Several other WNT ligands were differently expressed as well: WNT3 was upregulated, whereas WNT6, WNT10A and WNT11 were downregulated.

In general, an upregulation was seen in Wnt receptors, namely FZD2 and FZD3. Interestingly, several negative regulators are downregulated in nodule tissue of Dupuytren's patients: DKK1, FRZB, SFRP1 and WIF-1. Because of that, Wnt signaling is not inhibited and thus very active. For example, KREMEN1 is a transmembrane receptor that functionally cooperates with DKK1 to block Wnt/β-catenin signaling. In the absence of DKK1, KREMEN1 potentiates Wnt/β-catenin signaling by maintaining LRP5 or LRP6 at the cell membrane. [26] In nodules, we found that DKK1 is highly downregulated and that KREMEN1 is highly upregulated. FRZB, SFRP1 and WIF-1 binds to both β-catenin dependent and independent Wnt proteins, and prevents them from triggering signaling. The downregulation of DKK1, FRZB, SFRP1 and WIF-1 could activate both β-catenin dependent and independent signaling. mRNA and protein levels of Wisp-1, a known target of β-catenin dependent Wnt signaling, was indeed increased. NKD1, PRICKLE1 and VANGL2, components of the β-catenin independent pathway, were also found to be upregulated, as well as WNT5A, a Wnt protein usually associated with the β-catenin independent pathway. VANGL2 and Wnt5a were further studied with immunohistochemistry, and both showed a significant increase in nodule tissue compared with control tissue. The receptors Ror2 and Ryk, both used in the β-catenin independent pathway, were increased as well (as revealed by mRNA and protein levels, respectively).

Since TGF-β1 is one the most potent pro-fibrotic cytokines, and since TGF-β1 has repeatedly been reported to be increased in diseased Dupuytren's tissue, we investigated whether TGF-β1 is able to suppress the four mentioned negative regulators of Wnt signaling. Indeed, normal dermal fibroblasts exposed to TGF-β1 showed a sharp decrease in all of the mentioned negative regulators. The TGF-β pathway and Wnt pathway are thus intertwined.

In conclusion, we found a decrease a several negative regulators of the Wnt signaling pathway, most probably due to the increased levels of TGF-β in Dupuytren's tissues, leading to an activation of various pro-fibrotic elements of both the β-catenin dependent and independent pathway.

In **Chapter 4** molecular differences between cords and nodules in Dupuytren's disease are described. It has previously been stated that the nodule is the active fibrotic disease unit, and that the cord can be considered as the previously formed fibrotic, but now quiescent, tissue. [27-29] This was confirmed by our studies: we found significantly increased numbers of CD68-positive cells (mainly macrophages) and α-smooth actin positive cells (mainly myofibroblasts) in nodules. In addition, the number of proliferating cells (as revealed by Ki-67 staining) was increased in the nodule. We subsequently studied whether there are differences in extracellular matrix protein synthesis/deposition between nodules and cords. With respect to collagen types, a higher mRNA expression was found for COL1A1, COL1A2, COL5A1 and COL6A1 for nodules, whereas no differences in COL3A1 and COL4A1 expression was found between nodules and cords. From the five studied non-collagenous extracellular matrix molecules (BGN, DCN, ELN, FMOD and FN1), only the latter showed differences in mRNA levels between nodules and cords: it was elevated in nodules.

A different picture emerged with immunohistochemistry: increased deposition levels were seen for fibronectin, elastin, and collagen type V in nodules, whereas collagen type I was increased in cords. Clearly, the extracellular matrix composition between cords and nodules is different. This was substantiated by the increased Hyp/Pro ratio in cords, meaning that cords exhibit a higher abundance of collagen to non-collagenous proteins compared to nodules. The collagen in both tissues showed a high level of pyridinoline crosslinks, with the highest level in the cord. An elevated pyridinoline crosslink level in collagen is characteristic for fibrotic processes. Such collagen is difficult to degrade by the regular collagenases (MMP1, MMP8), but not by MM13 or cathepsin K.

Procollagen type I staining revealed a much more active synthesis of this protein in nodules than in cords, although the actual collagen deposition level in nodules is lower. This is probably due to the lower PCOLCE2 expression in nodules. This protein is a co-factor for BMP1, and assists with cleaving off the C-propeptide from procollagen by BMP1. The presence of the C-propeptide

hampers the deposition of collagen. Immunohistochemistry revealed normal levels of BMP1 in nodules, but decreased levels of PCOLCE2.

In conclusion, cords display a paucity of cells in an abundant extracellular matrix, pointing to a residual state of the cords. In contrast, active fibrogenic processes take place in the nodule. Furthermore, the composition of the extracellular matrix is different between cords and nodules.

Chapter 5 addresses the question whether inhibition of the Wnt signaling pathway may result in a less fibrotic phenotype in Dupuytren's disease. Since there are many Wnt ligands, we were looking for a compound that inhibits all Wnt ligands. For this purpose we have used IWP-2, a cell-permeable inhibitor of Wnt processing and secretion. This inhibitor acts on the protein porcupine, a membrane-bound O-acyltransferase, thereby preventing palmitylation of Wnt proteins. As a consequence, secretion of Wnt proteins is blocked, resulting in an inhibition of downstream Wnt signaling. Human dermal fibroblasts exposed to TGF-β1 were treated with IWP-2 showed a decrease in COL1A1, ACTA2 and TAGLN expression. Nodular fibroblasts from 2 patients were treated with IWP-2 as well: one patient showed a decrease in the expression of all fibrotic markers measured (COL1A1, COL5A1, ACTA and TAGLN), whereas the other patient did not react on the treatment. However, it should be stressed that the latter patient had a much lower expression of the four mentioned markers than the former patient. Both patients showed less nuclear localization of β-catenin, a sign of an inhibited Wnt signaling pathway.

In recent years, multiple Wnt targeted therapies have been studied. The use of mall molecules has shown effective results, some of them also targeting Porcupine, being IWP-2, C59, and LGK974. [11, 30, 31] Molecules that impact the stability of Axin, XAV939 and IWR, are also able to inhibit Wnt signaling. [32, 33] Although a large number of studies are focused on cancer treatment, fibrotic disorders that may benefit from these small molecule treatments, are skin fibrosis [31] and cardiac fibrosis. [34] Thus, targeting Wnt signaling is possible in multiple ways, and novel compounds will probably increase these numbers. The transition of results from experimental or animal models to human disease, however, will be one of the major challenges. Clinical trials on cancer, inhibiting Wnt signaling on either ligand secretion or receptor blocking, are underway, giving hope to comparable trials on fibrotic disorders in the near future. [9]

The last question of this thesis, namely is there – compared to Dupuytren's disease – a similar dysregulation in genes and pathways in plaques from Peyronie's disease patients, is investigated in **Chapter 6**. In this chapter we studied the presence of Wnt and Wnt-related proteins in plaques of the tunica albuginea from Peyronie's patients and compared it with unaffected tunica albuginea from the same patients. We stained for Wnt2, Wnt4, Wnt5a, Wnt7b and β-catenin. An increased staining was seen for Wnt4 and β-catenin only. The staining of Peyronie's plaques with regard to the Wnt ligands was thus completely different from that of Dupuytren's tissues. In the latter, a decreased staining was seen for Wnt2 in cords, an increased staining of Wnt5a and Wnt7b in nodules, and a normal level of Wnt4. All of the mentioned Wnt ligands have been connected to Dupuytren's disease, [1, 3, 4] but only the WNT2 locus seems to be involved in Peyronie's disease. [2] No increased staining was seen for Wisp-1; whereas we found increased levels in Dupuytren's nodules. Increased levels of Wnt4 have been associated with interstitial renal fibrosis; [35] its increase in Peyronie's plaques might thus facilitate fibrogenic processes. Intense staining was observed for YAP1, a transcription factor found to be involved in fibrosis and DD. [36]

As expected, the plaques contained more myofibroblasts, as revealed by an increased staining of α-smooth muscle actin and the increased mRNA levels of its gene ACTA2. The same applied to collagen type I and III. The collagen in the plaques is different from that of the unaffected part of the tunica albuginea. It has previously been found that the diameter of the collagen fibrils in plaques is smaller. [37] Collagen fibrils are of a heterotypic nature, i.e. they contain more than one collagen type. We observed an increased COL3A1 and COL5A1 mRNA ratio towards COL1A1 (i.e. an increased amount of collagen type III or V at the expense of collagen type I). This could explain the thinner fibrils, as there is an inverse relationship between the amount of collagen type III or V towards collagen type I. [38] Furthermore, the collagen in plaques is likely to be overmodified, as mRNA levels of PLOD3 were highly upregulated. The resulting enzyme, lysyl hydroxylase 3, converts lysine into hydroxylysine, on which sugars can be added. This overglycosylation of collagen is also known to lead to a decreased fibril diameter. Interestingly, overhydroxylation of lysine and a hydroxylysine overglycosylation has been reported for affected Dupuytren's tissues. [39, 40]

Importantly, a major decrease is seen in the expression of cathepsin K in plaques. This enzyme is able to degrade fibrillar collagens in an effective way, especially collagens that contain pyridinoline crosslinks (which is seen in

fibrotic collagen of Dupuytren's disease). The collagen accumulation in the plaque might be partially due to the decreased levels of cathepsin K.

We conclude that the cellular and extracellular matrix composition of the plaques is different from that of the unaffected tunica albuginea: there is an abundance of myofibroblasts which are YAP1 positive, there is an increased amount of collagen type I and III, the fibrils are different, and there is a paucity of cathepsin K. Remarkably, the Wnt signature in the plaques from Peyronie's patients is different from that from Dupuytren's patients, but this is in agreement with the GWAS data from Dolmans et al. [2]

Wnt signaling and cancer

In many cancer types, such as breast cancer, colon cancer and melanoma, Wnt related genes are involved. [41, 42] Although fibrotic diseases such as DD share some clinical features with cancer – extracellular matrix deposition, excess cellular growth and recurrence after treatment – fibrosis is essentially benign. As expected, there is a difference in expression between fibrotic (benign) and oncologic (malignant) diseases. In DD, dysregulation of the Wnt pathway is seen upstream of the receptor and thus receptor dependent. Interfering in Wnt signaling therefore shows promising results. [43] In cancer, dysregulation is seen downstream of the receptor, for example in β-catenin and APC gene mutations. [44] Being receptor independent, this downstream dysregulation might lead to unrestrained growth and proliferation. In that case, interfering with Wnt itself is unlikely to have an effect on the tumor. [11, 45]

Collagen processing

The essence of fibrotic conditions is the excessive accumulation of matrix proteins, especially collagen. This is explained by a decrease in the expression of collagenase genes, leading to an overproduction of collagen. In addition, the expression of collagen-degrading enzymes, such as matrix metalloproteinases (MMP1, MMP8, MMP13) and cathepsin K, is diminished, leading to lower collagen turnover. Furthermore, an increase is seen in the expression of proteins that inhibit MMPs, known as tissue inhibitors of metalloproteinases (TIMPs). [46] This results in an imbalance of collagen production/degradation, with massive collagen deposition.

Apart from the above, the collagen synthesis itself is a multistep process. During synthesis of collagen, various modifications take place, such as the hydroxylation of proline and lysine residues, and the addition of sugars on hydroxylysine. Procollagen, the precursor of collagen, is subsequently secreted by the cell, after which it is further processed. The propeptides are cleaved off, resulting in collagen. The collagen molecules aggregate into fibrils, and the molecules are connected to each other by means of crosslinks. All these steps are mediated by various enzymes. [47] Alterations in the expression of these enzymes leads to collagen that is qualitatively different from collagen as seen in normal tissues. For example, in fibrosis an increase is seen in pyridinoline crosslinks, making collagen difficult to degrade by collagenases. [48] However, such collagens can still be cleaved by MMP13 and cathepsin K.

Novel therapies

Traditional treatment of DD and PD is mainly surgical by either removing or transecting the affected tissue, but less invasive methods are upcoming. *Collagenase clostridium histolyticum* injections into the cords in DD and the plaques in PD dissolve the collagens that have been deposited, and may lead to a relieve of symptoms. [49] Since collagen deposition is a result of (myo)fibroblast overactivity, which results from aberrant inflammatory processes and Wnt signaling, it would make sense to interfere in those processes. Prevention of fibrosis and thus collagen deposition and contractures may have better outcomes than the treatment of these symptoms. Tumor necrosis factor, a cytokine involved in inflammation, has shown its ability to convert palmar fibroblasts from DD patients into myofibroblasts, but not the other fibroblasts. It functions through Wnt signaling. TGF-β, as previously mentioned, has a much stronger pro-fibrotic effect on all kinds of fibroblasts. [50] A trial with anti-TNF injections (adalimumab) in DD nodules shows a downregulation of the myofibroblast phenotype, a very promising result that is currently tested in the clinic. [51] Since TNF acts through the Wnt signaling pathway, and we showed the importance of Wnt signaling in DD and PD, Wnt inhibition is a clear possible target in these fibromatoses. Selective inhibition of Wnt signaling has been studied in fibrotic diseases, and IWP-2, the inhibitor we used to block Wnt in Chapter 5, seems capable of preventing cardiac remodeling and fibrosis after injury. [52] Similar effects are seen in oncology research, but to us it would be more interesting to see if more organ fibroses would respond to IWP-2 treatment as well.

Another way of thinking is to look at wound healing that does not lead to scar formation, like it is in fetuses. Unlike in adults, the wounded tissues in fetuses heal with a normal collagen pattern. This process does not depend on the fetal environment, so modifications in adult wound healing processes could result in beneficial outcomes. [53] Irresponsiveness to TGF-β stimulation and higher expression of MMPs is seen in fetal fibroblasts as compared to their adult counterparts, and the inflammatory response is almost absent. [54, 55] If we might be able to convert DD and PD processes to something more similar to fetal wound healing, we will probably see less or no fibrosis.

Society

Dupuytren's and Peyronie's diseases both are debilitating conditions for the patient. Since the prevalence can be very high, in Western countries ranging from 0,6 to 31,6%, [56] it has a great impact on society, as it comes to the ability to work and function on a normal basis. Since more fingers in both hands might be affected metachronically in DD, and recurrence is often seen, most patients need different procedures. The costs of these diseases are high due to medical treatment and a period of rehabilitation after surgery. [57-59] Life expectancy improves in many Western societies, and people are expected to work longer than they do right now. For example, in the Netherlands, the average age of retirement has risen from 61 to 65 years in less than two decades time, and this age will become even higher in the following years. [60] Disability to work in these years might become more evident due to this manual condition with a relatively high prevalence. Improvement in better-targeted therapies without recurrence can save society significant amounts of money.

Working together

The collaboration between different medical departments, here being Plastic Surgery, Urology and Medical Biology, has shown that looking at a disease from different angles can help medical professionals gaining more knowledge. The problems that patients report in the outpatient clinic, combined with treatment insufficiencies that medical doctors report, and a biological view on the processes that happen on a microscopic level, give rise to new insights and possible targets. Collagenase injections have been around for a while now, after long trials on the efficacy and safety in treating fibromatosis. Anti-TNF injections have shown hopeful results, as might selective Wnt blocking agents

in the future. Research is ongoing and at this moment, new studies are being performed at our departments. Young and ambitious biologists, medical doctors and PhD students are following up on the studies as described in this thesis.

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